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Pharmacological properties of centrally administered ouabain and their modification by other drugs

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Summary

1. Ouabain given by intracerebroventricular injection to mice in small doses (0·1–0·4 μg) produced a dose related depression of central nervous activity, characterized by a reduction in spontaneous locomotor activity, hypothermia, catalepsy and ptosis, lowered body posture and lack of response to external stimuli. Doses above 0·4 μg were excitatory, convulsant and lethal.

2. The depressant effects could be antagonized by (+)-amphetamine, desmethylimipramine, dibutyryl cyclic 3′5′-adenosine monophosphate and caffeine.

3. The MAO inhibitor nialamide produced only a small antagonism of ouabain, resulting in a greater rate of recovery from the depressant effects of ouabain.

4. The depressant effects were associated with a marked elevation of whole-brain dopamine levels with little change in noradrenaline or 5-hydroxytryptamine.

5. The dopamine-β-hydroxylase inhibitor sodium diethyldithiocarbamate, administered by intracerebroventricular injection, produced effects qualitatively similar to those seen after ouabain.

6. An interference with central transmitter function is postulated as a possible mode of action of intracerebroventricularly injected ouabain.

Introduction

Since Withering's classical observations in the eighteenth century, it has been repeatedly observed that the cardiac glycosides can produce central effects in man (Batterman & Gutner, 1948). As yet, it is not known whether these effects are mediated through a direct action on the central nervous system, or alternatively, whether they are behavioural manifestations of peripheral actions of these glycosides.

Many reports have appeared implicating catecholamines in both the therapeutic and toxic actions of the cardiac glycosides on the heart, and in some species, it has been shown that the cardiac glycosides can release endogenous catecholamines which act, for example, on the myocardium to produce their characteristic inotropic effects (Tanz, 1967). More recently, Hermansen (1970) has shown that the cardiotoxic effects of ouabain in guinea-pigs are brought about partially by a liberation of

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endogenous catecholamines. Furthermore, Butterbaugh & Spratt (1970) have suggested that the amine concentrations of intact brain have a role in the development of digitoxigenin toxicity.

Several recent studies have examined the pharmacological actions of substances injected directly into the brain by intracerebroventricular (i.c.v.) injection. In the mouse, for example, noradrenaline produces marked depression of central nervous activity when given by this route (Brittain & Handley, 1967). Clearly, if ouabain is capable of releasing noradrenaline from its storage sites within the brain, one would expect its central actions to resemble those of noradrenaline. However, observations to date, at least with toxic doses, show that ouabain causes death by convulsions (Greef & Kasperat, 1961a, b).

Accordingly, a study has been made of the pharmacological actions of ouabain given by intracerebroventricular injection in the mouse. The mouse was chosen since intracerebroventricular injection is relatively simple in this animal and a rodent was the species of choice in view of the relative resistance of the heart to the effects of cardiac glycosides (Seager, Murphee & Munroe, 1965). A preliminary report of this work has been given to the British Pharmacological Society (Doggett, Spencer & Turner, 1970).

Methods

Animals

Male albino mice of a TO strain, weighing 16–20 g, were housed under constant environmental conditions of $21 \pm 1°$ C (or $32 \pm 1°$ C where stated) at a relative humidity of about 60%. They were allowed free access to drinking water and a conventional 41 B cube diet until 2 h before experiment, when both were withdrawn.

Injections

Intracerebroventricular injections were made according to the method of Haley & McCormick (1957), as modified by Brittain & Handley (1967). Unless otherwise stated, ouabain and all other agents given by intracerebroventricular injection were dissolved in 0·9% w/v sterile apyrogenic NaCl solution and administered in a dose of 10 μl. Agents given by peripheral injection were dissolved in 0·9% w/v NaCl solution and administered in a dose of 10 ml/kg.

Measurement of body temperature

Oesophageal temperatures were determined using the method of Brittain & Spencer (1964); a thermocouple, attached to a graduated electric thermometer (Light Laboratories, Brighton, England), was inserted into the oesophagus of the mouse to a depth of about 2 cm so that its tip lay just above the cardia of the stomach. Skin temperatures were determined in still air by placing a flattened thermocouple between the digits of the right ventral hind paw, the mouse meanwhile standing upon a sheet of expanded polystyrene foam-insulation to minimize heat loss by conduction; skin temperatures were measured on the same calibrated thermometer, the mice being housed individually for the duration of the experiment.

Spontaneous locomotor activity

In most experiments, the assessment of locomotor activity changes and general central nervous activity was made by direct visual comparison of test and control groups. On two occasions, a more quantitative evaluation of locomotor activity was desirable, when spontaneous locomotor activity was measured using a Faraday Electronics Animal Activity Recorder. Two groups of five mice (test and control) were placed in opaque polypropylene cages measuring 10 cm × 27 cm. A high frequency radio signal was fed on to a steel grid under each cage and movement of the mice above these grids altered the capacitance of receiver plates in the sides of both cages. The signals from these plates were fed into two balanced integrating amplifiers, so that for each group of mice, there was displayed a digital assessment of locomotor activity.

Production of electro-convulsive shock

Electro-shock convulsions were produced by a modification of the method of Cashin & Jackson (1962) using a constant voltage square-wave stimulator (Scientific and Research Instruments Ltd., London). For administration of the shock the ears of the mice were filled with 0·9% w/v NaCl solution (wetted with 0·5% v/v Tween 80), and the mice held with the electrodes placed in the ears. Tonic extensor spasm could be induced in 30% of control animals at 65 V using a pulse rate of 100 Hz, a pulse width of 2 ms and pulse duration of 0·3 seconds.

Determination of tissue amine concentrations

Whole brain catecholamine and indoleamine concentrations were determined spectrophotofluorimetrically using the methods of extraction and assay described by Spencer & Turner (1969).

Results

Pharmacological effects of intracerebroventricular ouabain

Under normal laboratory conditions (21° C and relative humidity of 60%), groups of five conscious mice were given intracerebroventricular injections of ouabain, 0·1–0·4 μg per mouse, and the animals were observed continuously for the next 5–6 h for changes in motor activity, body temperature and general behaviour.

Ouabain produced a profound, dose related depression of central nervous activity, characterized by a loss of spontaneous locomotor activity (Fig. 1a), hypothermia (Fig. 2a), ptosis, lowered body posture and tone, and failure to respond to external stimuli such as sound, touch or tail-pinch. Thirty minutes after 0·2 or 0·4 μg doses, the animals were markedly cataleptic. The whole body hypothermia was preceded by a sudden, transient increase in skin temperature (Fig. 3a), indicative of peripheral vasodilatation.

When the experiments were repeated at a raised environmental temperature (32° C), there was substantially less hypothermia (Fig. 2b), but there was still marked central nervous depression (Fig. 1b).

At doses of 0·1–0·4 μg intracerebroventricularly, ouabain had no anticonvulsant activity against convulsions produced by electro-shock; instead it tended to

enhance convulsions (Table 1). At doses in excess of 0·4 μg intracerebroventricularly, ouabain alone caused tonic or clonic convulsive phases in a proportion of mice; above 1·0 μg of ouabain, convulsions and death were the predominant behavioural effects.

Experiments were also performed in which the ouabain was given by a peripheral route of administration. Intravenous doses of ouabain up to 1·0 μg and subcutaneous doses up to 20 μg per mouse produced no signs of central nervous depression; occasionally, the largest doses produced a small degree of hypothermia (up to 3° C at 30 min).

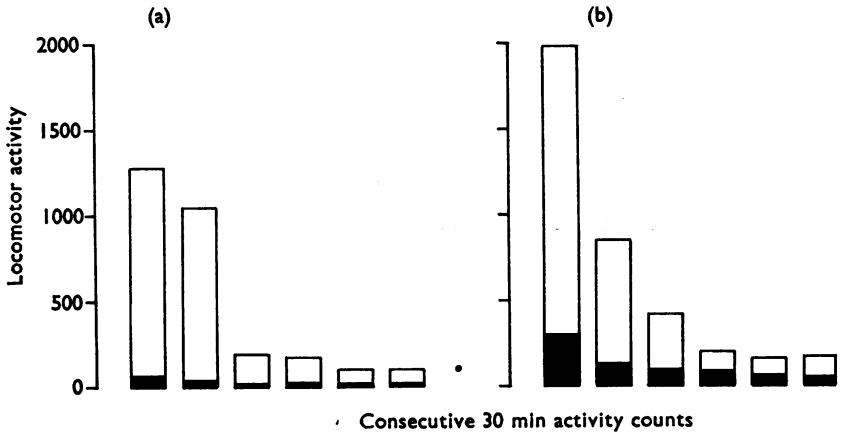

FIG. 1. Decrease in locomotor activity produced by intracerebroventricular injection of ouabain (a) at 20° C; (b) at 32° C. Mice received 10 μl saline (open columns) or 0·3 μg ouabain (solid columns).

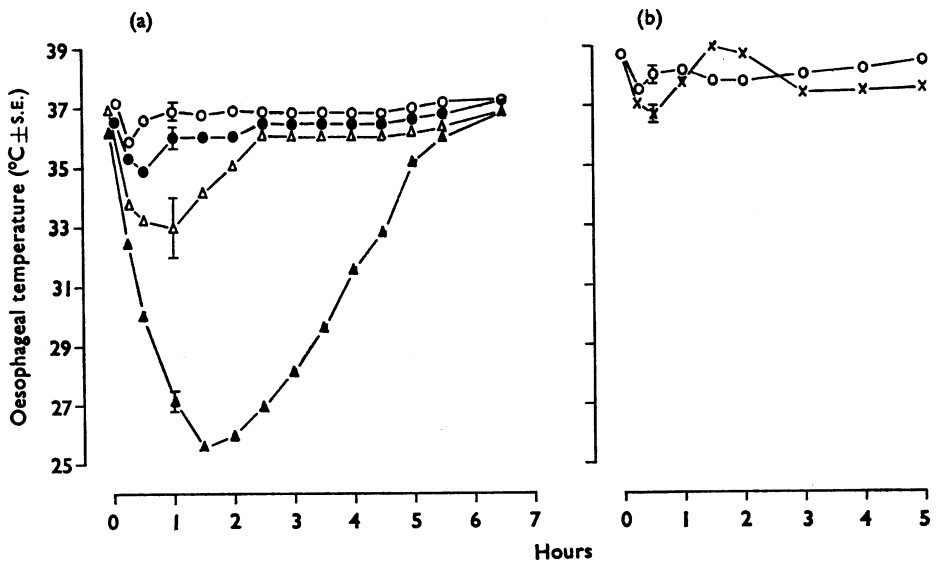

FIG. 2. Effect of intracerebroventricular injection of ouabain on body temperature at different ambient room temperatures (a) 20° C; (b) 32° C. (○——○), Controls; (●——●), ouabain 0·1 μg; (△——△), ouabain 0·2 μg; (×——×), ouabain 0·3 μg; (▲——▲), ouabain 0·4 μg.

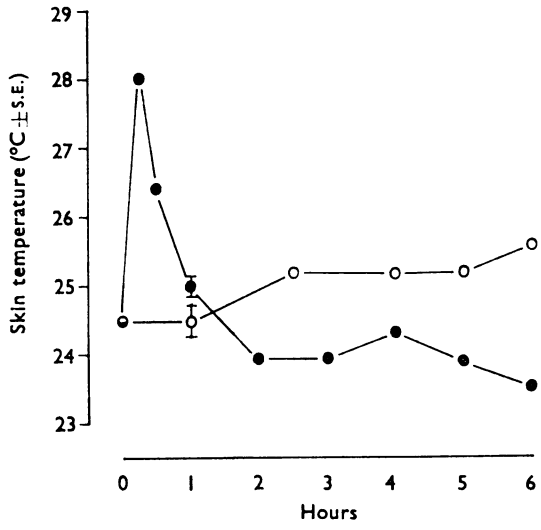

FIG. 3. Effect of intracerebroventricular injection of ouabain on skin temperature of mice at an ambient temperature of 20° C. (○——○), 10 μl saline ; (●——●), ouabain 0·4 μg.

TABLE 1. *Convulsion potentiating activity of intracerebroventricular ouabain following electro-shock*

Dose of ouabain (μg/mouse)	% Showing clonic extensor spasm	% Showing tonic extensor spasm	% Mortality
0	70	30	10
0·05	70	30	0
0·1	40	60	20
0·2	10	90	60
0·4	20	80	60

Animals received saline or ouabain 30 min before the application of electro-shock (2 ms pulses, 100 Hz for 0·3 s at 65 V).

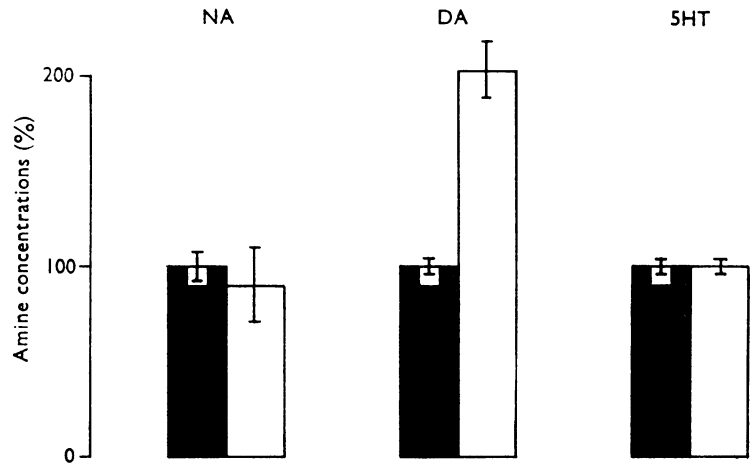

FIG. 4. Mice brain amine concentrations following intracerebroventricular ouabain. Determinations made on whole brains, removed 90 min after intracerebroventricular injection of 10 μl saline (solid columns) or 0·3 μg ouabain (open columns).

Effects of intracerebroventricular ouabain on whole brain amine concentrations

There have been several reports that both *in vitro* and *in vivo*, ouabain may interfere with the synthesis (Anagnoste & Goldstein, 1967 ; Goldstein, Ohi & Backstrom, 1970) and uptake (Berti & Shore, 1967 ; Blackburn, French & Merrills, 1967 ; Bogdanski, Tissari & Brodie, 1968) of catecholamines and indolealkylamines. Consequently, the effects of intracerebroventricular ouabain on whole brain amine concentrations were investigated in an attempt to explain some of the observed pharmacological effects of intracerebroventricular ouabain, described above.

Whole brain concentrations of dopamine, noradrenaline and 5-hydroxytryptamine were determined in mice killed 90 min after the intracerebroventricular injection of ouabain, 0·3 μg per mouse ; control mice received an equivalent amount of vehicle (10 μl i.c.v.). The results are summarized in Fig. 4.

The concentration of 5-hydroxytryptamine remained unchanged, but there was a highly significant increase in whole brain dopamine concentrations after ouabain pretreatment (103% increase ; $P=<0{\cdot}01$), together with a small, non-significant decrease in the concentration of noradrenaline.

Interaction of intracerebroventricular ouabain with other agents

The pharmacological effects of intracerebroventricular ouabain in the conscious mouse show marked similarities to the effects produced by peripherally administered reserpine or chlorpromazine, or by intracerebroventricularly administered noradrenaline. Although the whole brain amine determinations above indicate that ouabain elicits its effects through a mechanism different from that of either reserpine or chlorpromazine, nevertheless it seemed important to examine the interaction of intracerebroventricular ouabain with other agents known to interfere with central adrenergic function. Accordingly, a study was made of the pharmacological effects of intracerebroventricular ouabain in the presence of several of these agents given by peripheral or concomitant intracerebroventricular injection.

(+)-Amphetamine, 0·5 and 10 mg/kg intraperitoneally, produced a dose related reversal of all of the central nervous depressant effects of intracerebroventricular ouabain, 0·4 μg, when given 30 min after the ouabain ; the reversal of ouabain-induced hypothermia is illustrated in Fig. 5a. In another experiment, desmethylimipramine, 5 and 10 mg/kg intraperitoneally, was given 5 min before the intracerebroventricular ouabain, 0·3 μg. There was a marked reduction in all of the central nervous depressant effects of ouabain ; Fig. 5b summarizes the effects of desmethylimipramine pretreatment on the intracerebroventricular ouabain-induced hypothermia. In contrast, pretreatment with nialamide, 10 or 20 mg/kg intraperitoneally, 2 h before intracerebroventricular ouabain, 0·3 μg, failed to reduce or retard the development of central nervous depressant effects, although the rate of recovery from the ouabain effect was significantly enhanced by the MAO inhibitor, ($P=<0{\cdot}05$).

Abdulla & Hamadah (1970) have shown that the successful treatment of clinical depression with antidepressant drugs was accompanied by an increase in urinary concentrations of cyclic 3′,5′-AMP(cAMP) ; since intracerebroventricular ouabain produces, in mice, central nervous depressant effects which are similar to those of reserpine, and these same workers claimed reserpine ptosis can be reversed by dibutyryl cyclic 3′,5′-AMP(dbcAMP), an attempt was made to antagonize the de-

pressant effects of intracerebroventricular ouabain with concomitantly administered dbcAMP ; this agent was used because it can cross cell membranes more readily than its analogue cAMP (Butcher & Sutherland, 1962, 1967), and its rate of hydrolysis by phosphodiesterase is very much slower than that of cAMP (Moore, Iorio & McManus, 1968). Groups of five mice were given, by intracerebroventricular injection, ouabain alone, 0·3 μg, dbcAMP alone, 25 μg, or a mixture of both in the same injection. (Preliminary examination of this mixture by infrared and ultraviolet spectroscopy had shown that there was no chemical interaction between these two agents). Control animals received the vehicle only, 10 μl, by intracerebroventricular injection.

Compared with control animals, the intracerebroventricular injection of dbcAMP alone produced no overt changes in behaviour, and body temperature remained similar to that of untreated and control animals. Whilst intracerebroventricular ouabain produced the expected depression of central nervous activity, there was a clear reduction in the level of depression when animals were given the mixture of ouabain and dbcAMP ; animals were noticeably more mobile, they were not cataleptic, were more sensitive to touch, sound and painful stimuli, and the level of hypothermia was reduced (Fig. 6a).

In view of the observed antagonism of ouabain's effects by dbcAMP, the effect of caffeine was also studied because of its ability to inhibit the enzyme phosphodiesterase. Two experiments were performed ; in the first, doses of caffeine of 20, and 40 mg/kg were given intraperitoneally at the same time as the intracerebroventricular injection of ouabain, 0·25 μg. There was a substantial antagonism

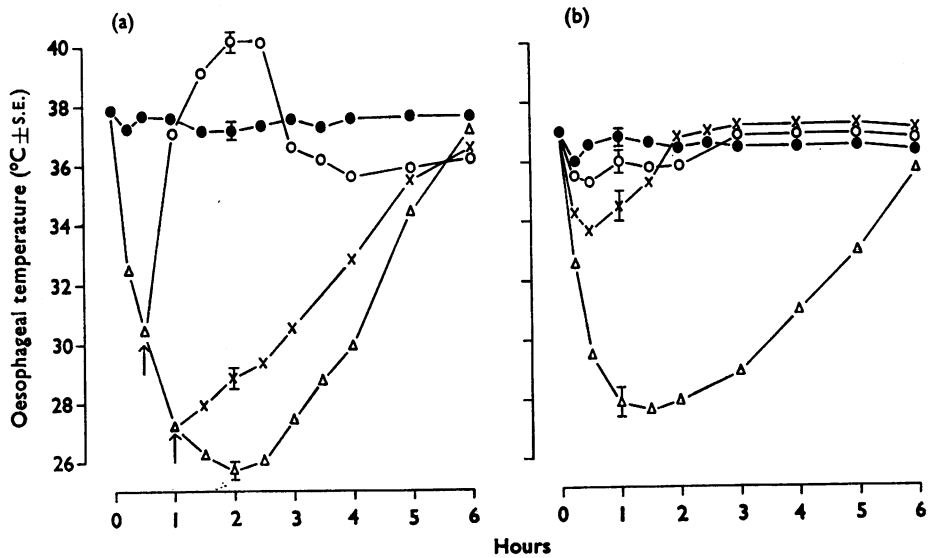

FIG. 5. Effect of (+)-amphetamine and desmethylimipramine on ouabain-induced hypothermia, (a), (●——●) 10 μl saline intracerebroventricularly ; (○——○), 0·4 μg ouabain intracerebroventricularly+10 mg/kg (+)-amphetamine intraperitoneally at the arrow ; (×——×), 0·4 μg ouabain intracerebroventricularly+0·5 mg/kg (+)-amphetamine intraperitoneally at the arrow ; (△——△), 0·4 μg ouabain intracerebroventricularly+10 ml/kg saline intraperitoneally. (b), (●——●), 10 μl saline intracerebroventricularly ; (×——×), 5 mg/kg and (○——○), 10 mg/kg desmethylimipramine intraperitoneally 5 min before ouabain 0·3 μg intracerebroventricularly at time 0 ; (△——△), 0·3 μg ouabain intracerebroventricularly.

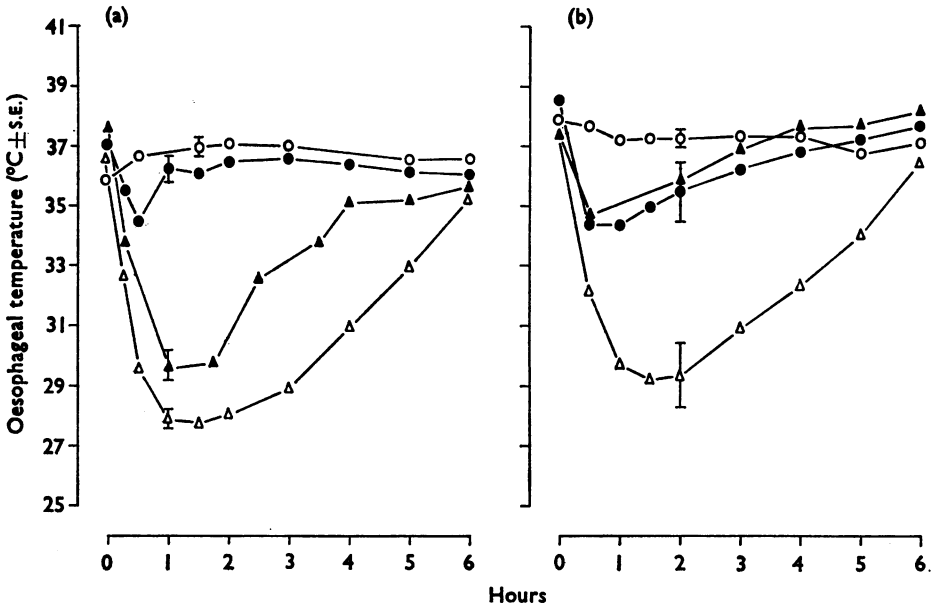

FIG. 6. Effect of dibutyryl cyclic 3′,5′-AMP and caffeine on ouabain induced hypothermia. (a), (○——○), 10 μl saline intracerebroventricularly; (●——●), 25 μg dbcAMP intracerebroventricularly; (▲——▲), 25 μg dbcAMP+0·3 μg ouabain intracerebroventricularly; (△——△), 0·3 μg ouabain intracerebroventricularly. (b), (○——○), 10 μl saline intracerebroventricularly; (●——●), 0·25 μg ouabain intracerebroventricularly+20 mg/kg caffeine intraperitoneally; (▲——▲), 0·25 μg ouabain intracerebroventricularly+40 mg/kg caffeine intraperitoneally; (△——△), 0·25 μg ouabain intracerebroventricularly+10 ml/kg saline intraperitoneally.

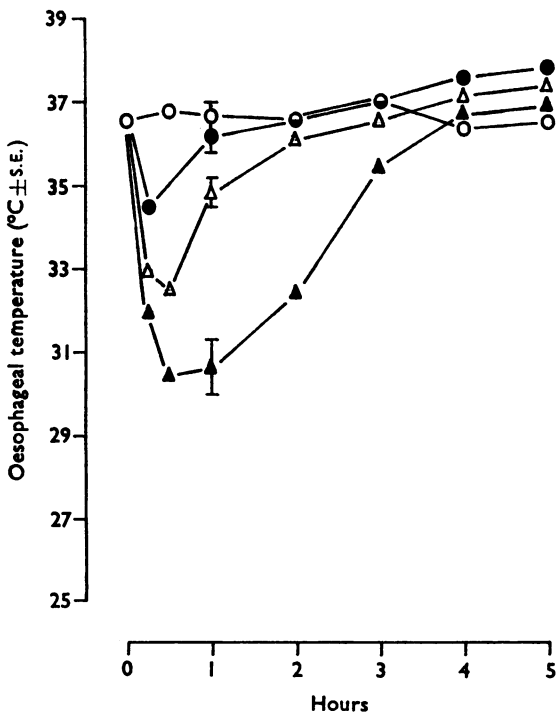

FIG. 7. Effect of intracerebroventricular injection of sodium diethyldithiocarbamate on body temperature in mice at an ambient temperature of 20° C. Mice received by intracerebroventricular injection: (○——○), 10 μl saline; (●——●), 0·2 mg; (△——△), 0·5 mg; (▲——▲), 1 mg sodium diethyldithiocarbamate.

of the central nervous depressant effects of ouabain, with ptosis, lack of reactivity and hypothermia (Fig. 6b) all substantially less than that seen with intracerebroventricular ouabain alone. In the second experiment, caffeine, 75 mg/kg intraperitoneally, was given 30 min before the intracerebroventricular ouabain, 0·25 μg. There was a marked reduction, but not complete prevention, of the central nervous depressant effects of intracerebroventricular ouabain; maximum hypothermia (at 60 min) was 3·7° C as opposed to 7·2° C in animals treated with ouabain only, a highly significant reduction ($P=<0{\cdot}001$).

Effects of intracerebroventricular sodium diethyldithiocarbamate (DDC)

Finally, the effects of DDC on body temperature and behaviour were studied. DDC is a potent inhibitor of dopamine-β-hydroxylase in brain and other tissues (Carlsson, Linquist, Fuxe & Hökfelt, 1966). It has been suggested that the behavioural changes associated with centrally administered DDC are dependent on the depletion of brain noradrenaline or on the increase of brain dopamine. (Kleinrok, Zebrowska & Wielosz, 1970). Consequently it seemed of interest to compare DDC with ouabain.

The effects of intracerebroventricular DDC were qualitatively similar to those observed after intracerebroventricular ouabain; the animals showing catalepsy and ptosis and a reduction in locomotor activity. Body temperature was also lowered in animals maintained at a laboratory temperature of 20° C, the effect lasting from 15 min to 3 h after injection. (Fig. 7). All these effects were dose dependent. Some of the animals receiving the highest dose of DDC (2 mg i.c.v.) exhibited tonic convulsions immediately after injection, death occurring in 40% of the animals receiving this dose.

Discussion

The intracerebroventricular injection of small doses of ouabain into conscious mice produces a profound central nervous depression, characterized by a marked loss of spontaneous locomotor activity, catalepsy, ptosis and poikilothermia. These effects are reminiscent of those produced by peripherally administered reserpine, suggesting that ouabain too may interfere in some way with central aminergic function. The observation that spontaneous activity could be reduced and catalepsy induced by ouabain in the absence of hypothermia (experiments conducted at 32° C) confirms that, although inter-related, temperature changes and behavioural changes are essentially separately induced effects. In this respect, our observations with ouabain confirm earlier observations in these laboratories using chlorpromazine and reserpine (Spencer & Waite, 1968). The intracerebroventricular injection of larger doses of ouabain (above 0·4 μg/mouse) was excitatory, and caused death by convulsions, thus confirming the observations of Greef & Kasperat, (1961a, b).

In contrast, ouabain administered by peripheral injection was not accompanied by any gross change in behaviour; there was no catalepsy, no measurable reduction in locomotor activity and no ptosis, and a slight hypothermia was limited to 1 or 2° C at 30 minutes. This general lack of depressant (or excitant) activity after peripheral injection is taken to indicate that the effects of small doses of intracerebroventricular ouabain are mediated at a central level.

Ouabain differs from reserpine in one important aspect; it did not reduce whole brain concentrations of the amines noradrenaline, dopamine or 5-hydroxytryptamine at doses which produced marked central nervous depression; in fact, ouabain, 0·3 μg intracerebroventricularly per mouse, caused a marked increase (103%) in whole brain dopamine concentrations ($P=<0{\cdot}01$).

Despite this apparent difference between reserpine and intracerebroventricular ouabain, there are several similarities in their effects upon behaviour, and this prompted a study of the interactions of intracerebroventricular ouabain with agents known to alter the effects of reserpine, namely (+)-amphetamine, desmethylimipramine and the MAO inhibitor nialamide. Like reserpine, intracerebroventricular ouabain appears to sensitize mice to the effects of (+)-amphetamine, small doses of the latter promptly and completely reversing all of the behavioural changes induced by i.c.v. ouabain. Similarly, desmethylimipramine was able to prevent and reverse the effects of ouabain. Yet, the MAO inhibitor nialamide was devoid of anti-ouabain activity at doses up to 20 mg/kg. This last observation suggests that any interference with transmitter function by intracerebroventricular ouabain occurs at a cell membrane or extracellular site, two areas in which amine function is not likely to be affected markedly by inhibition of MAO. It is interesting to note here that Barnett & Taber (1968) found a similar lack of effect with the MAO inhibitor pargyline on the hypothermia produced by DDC.

Thymoleptics which interfere with amine uptake mechanisms, for example, protryptyline (Persson & Waldeck, 1968) and desmethylimipramine (Nyback & Sedvall, 1968), also enhance the synthesis of dopamine and reduce that of noradrenaline in the CNS. It is also known that ouabain can block the sodium pump through an action on the Na-K ATPase activity, and Tarve & Brechtlová (1967) have shown that imipramine can inhibit the Na-K ATPase system in guinea-pig brain microsomes. Recently, Ebadi & Carver (1970) have shown that chlorprothixene has similar effects in the rat brain. These observations, together with the knowledge that the reuptake of neuronally released catecholamines is inhibited by blockade of the sodium pump (Berti & Shore, 1967), suggest that the pharmacological effects of intracerebroventricular ouabain may be brought about by an interference with amine reuptake in the brain. However, such a theory is difficult to reconcile with the anti-ouabain effects of desmethylimipramine unless, because of its peripheral (subcutaneous) administration, the latter exerts its anti-ouabain effects at another site.

Nevertheless, our observations with DDC support the view that an interference with dopamine metabolism might be the single most important factor in the mechanism of action of intracerebroventricular ouabain. Recently, Kleinrok *et al.* (1970) have shown in rats that DDC-induced motor inactivity and hypothermia coincides with maximum decreases in noradrenaline and increases in dopamine concentrations in the brain. Anagnoste & Goldstein (1967) have shown that ouabain can enhance the synthesis of dopamine and reduce that of noradrenaline in the CNS; whilst this might confirm our observed increase in whole brain dopamine concentrations, their work might also predict an associated reduction in noradrenaline concentrations which we have not observed following intracerebroventricular ouabain. It seems likely that we cannot adequately interpret the action of ouabain from observations on whole brain amine concentrations since the behavioural effects witnessed here may be dependent upon other effects produced

only within the periventricular tissues (for example at the hypothalamic level) and not detected by whole brain determinations ; further work here is indicated.

The experiments with dbcAMP and caffeine were prompted by the experiments of Abdulla & Hamadah (1970), in which they claimed that the successful treatment of clinical depression by thymoleptics was associated with an increased urinary excretion of cAMP. Field, Plotkin & Silen (1968) have demonstrated an antagonism between the cardiac glycosides and cAMP on ion permeability. Thus, the reduction in intracerebroventricular ouabain-induced central nervous depression by dbcAMP and caffeine in our experiments may be produced by antagonizing an effect of ouabain on ion transport in the cell membrane. An alternative explanation, at least for caffeine, stems from the observation by Berkowitz, Tarver & Spector (1969) that methylxanthines can release catecholamines from central nervous stores, an effect not attributable to an inhibition of the enzyme phosphodiesterase.

The data provided in this paper do no more than outline the pharmacological effects of ouabain in central nervous tissue. Because of the known effects of ouabain and related cardiac glycosides on cell membranes, ion transport, and so on, a number of tentative explanations of intracerebroventricular ouabain's effects have been put forward ; at this stage it is difficult to reconcile its observed pharmacological effects with any one single mode of action. Whether the interference with transmitter function is pre- or postsynaptic, or a combination of both, must await further investigation. In view of the definite antagonistic effects of centrally acting sympathomimetics and thymoleptics, coupled with an overall similarity to reserpine in its behavioural effects (in the absence of peripheral actions), intracerebroventricular ouabain might prove to be a satisfactory alternative to reserpine in the experimental evaluation of certain classes of anti-depressant drugs.

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